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L14: Entry 5 of 6 File: USPT Jun 25, 1991

DOCUMENT-IDENTIFIER: US 5026644 A
TITLE: Process for preparing a lipid composition having a high .gamma.-linolenic acid content

ABPL:
A lipid composition having a high .gamma.-linolenic acid content is prepared by culturing mold fungi of the genus Cunninghamella in an aqueous nutrient culture medium having a relatively high concentration of a carbon source, and the lipid composition is recovered from the cultured mold fungi.

BSPR:
culturing mold fungi of the genus Cunninghamella in an aqueous nutrient culture medium containing a relatively high concentration of a carbon source; and

DEPR:
In order to determine which strains satisfy the conditions of a high lipid content, a high GLA content in the lipid and a fast growth, the present inventors screened strains from a catalogue. As a result of this screening, it was found that Cunninghamella elegans (to be referred to as C. elegans hereinafter; accession numbers NRRL-1378, 1379, 1380 and 1381) best satisfied these requirements. As a result of further studies made on the basis of this finding, it was also found that mold fungi of the genus Cunninghamella other than C. elegans substantially satisfy the above conditions and that culturing of such mold fungi in a specific culture medium can yield a lipid composition having a high GLA content. The present invention has thus been established.

DEPR:
These mold fungi can be generally cultured by static culture, shaking culture or aerated stirring culture using a liquid culture medium. A culture medium to be used is limited only in that it must contain carbon and nitrogen sources. However, a culture medium containing a relatively high concentration of a carbon source is preferably used.

DEPR:
An organic carbon source such as glucose or sodium acetate is preferable. Such a carbon source is contained, preferably, in the amount of 3 to 20% by weight based on the total weight of the culture medium. More preferably, the culture medium contains such a carbon source in the amount of 5 to 15% by weight.

DEPR:
A nitrogen source may be an organic nitrogen source such as yeast extract, malt extract, peptone, or urea; or an inorganic nitrogen source such as a nitrate or ammonium sulfate. Preferably, the nitrogen source is contained in the amount of 0.5 to 2% by weight based on the total weight of the culture medium.

DEPR:
As has been described earlier, the culture medium to be used herein is an aqueous liquid culture medium. This liquid culture medium can be prepared by dissolving the carbon and nitrogen sources in water. Preferably, the liquid culture medium is weakly acidic or neutral (pH 4.0 to 6.0). When vitamins such as vitamin B6 or biotin are added in the culture medium, growth of mold fungi

is facilitated. It is preferred that vitamin B6 and biotin are added to the medium in the amounts of 0.1 to 0.5 mg % and 0.001 to 0.005 mg %, respectively. Other source elements may be contained in the medium. Such source elements include a phosphorus source (e.g., potassium dihydrogen phosphate), a sodium source (e.g., sodium chloride), a magnesium source (e.g., magnesium sulfate), an iron source (e.g., ferrous sulfate), a calcium source (e.g., calcium chloride), a copper source (e.g., cupric sulfate), a zinc source (e.g., zinc sulfate), or a manganese source (e.g., manganese chloride).

DEPR:
When mold fungi of the genus Cunninghamella are cultured, using a culture medium as described above, the mold fungi are generally inoculated in the amount of 0.5 to 5 grams per liter of the medium. Culturing is preferably performed within a temperature range of 15.degree. to 30.degree. C. The culture period is 4 to 15 days.

DEPR:
The mold fungi cultured in this manner are recovered by filtering and the lipid content is extracted from the recovered mold fungi. Since the lipid containing GLA is not secreted in the medium during culturing, the culture medium need not be recovered.

DEPR:
An aqueous organic nutrient culture medium having the composition shown in Table A below was prepared.

DEPR:
0.2 grams of C. elegans (NRRL-1378) were inoculated in one liter of this medium and incubation was performed by shaking culture, which was done by horizontal turning at 100 rpm at 27.degree. C. for 5 days. After culturing, the mixture was filtered to recover the fungi which were freeze-dried at -30.degree. C. 2.5 grams of dried fungi were obtained per liter of the medium. The mold fungi were mixed with 20 grams of a solvent mixture of n-hexane and isopropanol in the ratio of 3:2 (Vol./Vol.) and the mixture was vigorously stirred at 10.degree. C. The solvent phase was recovered by filtering and the solvent was distilled off by reduced pressure distillation. 0.4 grams of the lipids were thus obtained.

DEPR:
An aqueous culture medium as shown in Table C below was prepared.

DEPR:
1.0 gram of the C. elegans (NRRL-1378) was inoculated in one liter of this culture medium and incubation was performed by shaking culture at 23.degree. C. and 100 rpm for 6 days. After culturing, the mixture was processed in the same manner as in Example 1 to yield 3.2 grams of freeze-dried fungi. The fungi were extracted in the same manner as in Example 1 to yield 0.55 grams of lipids. The fatty acid composition in the lipids was analyzed following the procedures of Example 1, and the obtained results are shown in Table D below.

DEPR:
An aqueous culture medium having the composition in Table E below was prepared.

DEPR:
Thirty liters of this culture medium were charged in a jar fermentor and sterilized at a temperature of 120.degree. C. and at a pressure of 1.5 kg/cm.sup.2. Thereafter, 30 grams of C. elegans (NRRL-1378) were inoculated in the medium and air-blowing stirring culture was performed at 28.degree. C. for 5 days. During culturing, 2N NaOH aqueous solution was added to maintain the pH of the medium at 4.0 or higher.

DETL:
TABLE A _____ (Culture Medium Composition)

Yeast extract 2 g/l Malt extract 3 g/l Peptone 3 g/l Glucose 50 g/l Water Balance to prepare 1 liter of composition

DETL:
TABLE C (Culture Medium Composition)
Yeast extract 2 g/l Ammonium sulfate 1 g/l Glucose 70 g/l Vitamin B6 2 mg/l Biotin 0.02 mg/l Water Balance to prepare 1 liter of composition

DETL:
TABLE E (Culture medium compostion)
Glucose 150 g/l Ferrous sulfate.7H.sub.2 O 20 mg/l Yeast extract 1 g/l Calcium chloride 20 mg/l Malt extract 1 g/l Cupric sulfate.5H.sub.2 O 0.5 mg/l Urea 5 g/l Zinc sulfate.7H.sub.2 O 3 mg/l Ammonium sulfate 5 g/l Manganese 3 mg/l chloride.4H.sub.2 O Potassium dihydrogen 5 g/l Vitamin B6 5 mg/l phosphate Magnesium sulfate 1 g/l Biotin 0.05 mg/l Sodium chloride 0.3 g/l Water (Balance to prepare 1 liter of composition)

CLPR:
2. A process according to claim 1, wherein the culture medium contains 3 to 20% by weight of the carbon source based on a total weight thereof.

CLPR:
4. A process according to claim 2, wherein the culturing step is performed at a temperature of 15.degree. to 30.degree. C. and with a weakly acidic or neutral culture medium.

CLPR:
5. A process according to claim 4, wherein the culturing step is performed by shaking culture.

CLPR:
6. A process according to claim 4, wherein the culturing step is performed by air-blowing stirring culture.

CLPR:
12. A process according to claim 11, wherein the culture medium contains 3 to 20% by weight of the carbon source based on a total weight thereof.

CLPR:
14. A process according to claim 12, wherein the culturing step is performed at a temperature of 15.degree. to 30.degree. C. and with a weakly acidic or neutral culture medium.

CLPR:
19. A process according to claims 1 or 11, wherein said culture medium contains 0.5% to 2.0% by weight of a nitrogen source.

CLPR:
24. A process according to claims 1 or 11, wherein said culture medium contains 0.1 mg % to 0.5 mg % of biotin.

CLPR:
25. A process according to claims 1 or 11, wherein said culture medium contains 0.001 mg % to 0.005 mg % of biotin.

CLPR:
27. A process for preparing a lipid composition having a high .gamma.-linolenic acid content of at least 20% comprising: culturing mold fungi of the genus Cunninghamella in an aqueous nutrient culture medium having a carbon source selected from the group consisting of glucose and sodium acetate; and recovering the lipid composition from the cultured mold fungi,

said lipid composition containing at least 20% .gamma.-linolenic acid.

CLPR:
28. A process for preparing a fatty acid composition containing at least 20% .gamma.-linolenic acid comprising: culturing mold fungi of the Cunninghamella genus in an aqueous nutrient culture medium containing a carbon source selected from the group consisting of glucose and sodium acetate; recovering the lipid composition form the cultured mold fungi, said lipid composition containing at least 20% .gamma.-linolenic acid; saponifying the recovered lipid composition to liberate a fatty acid composition containing at least 20% .gamma.-linolenic acid; and recovering the fatty acid composition.

CLPR:
29. The process of claims 27 or 28 wherein the culture medium contains 3-20% by weight of the carbon source based on the total weight thereof.

CLPR:
30. The process of claim 29 wherein the culturing step is performed at a temperature of 15.degree.-30.degree. C. and with a weakly acidic or neutral culture medium.

CLPR:
31. The process of claim 30 wherein the culturing step is performed by shaking culture.

CLPR:
32. The process of claim 30 wherein the culturing step is performed by air-blowing stirring culture.

CLPR:
37. The process of claims 27 or 28 wherein the culture medium contains 0.5% to 2.0% by weight of a nitrogen source.

CLPR:
42. The process of claims 27 or 28 wherein said culture medium contains 0.1 mg % to 0.5 mg % of biotin.

CLPR:
43. The process of claims 27 or 28 wherein said culture medium contains 0.001 mg % to 0.005 mg % of biotin.

CLPV:
culturing mold fungi of the genus Cunninghamella in an aqueous nutrient culture medium having a relatively high concentration of carbon source of 3-20% by weight, said carbon source being an organic source of carbon assimilable by the fungi and capable of producing fatty acids containing at least 20% .gamma.-linolenic acid when assimulated by the fungi in the nutrient medium; said culturing being performed under weakly acidic or neutral conditions; and

CLPV:
culturing mold fungi of the Cunninghamella genus in an aqueous nutrient culture medium containing a relatively high concentration of a carbon source of 3-20% by weight, said carbon source being an organic source of carbon assimilable by the fungi and capable of producing fatty acids containing at least 20% .gamma.-linolenic acid when assimilated by the fungi in the nutrient medium; said culturing being performed under weakly acidic or neutral conditions;

L14: Entry 3 of 8 File: USPT Jul 11, 1995

DOCUMENT-IDENTIFIER: US 5432064 A
TITLE: Process for dephosphorylating linear polynucleotide substrate with prosphatase form aspergillus niger

BSPR:
In accordance with the present invention, a purified heat-labile phosphatase enzyme from the filamentous fungus Aspergillus niger is provided. This enzyme has a native molecular weight of approximately 80,000 daltons, and, under denaturing conditions, consists of two identical polypeptide subunits, i.e., an alpha-2 dimer, each subunit having a molecular weight of approximately 37,000 daltons. In determining the native molecular charge of this protein, it is found in electrofocusing studies to have an isoelectric point (a "pI") of approximately 4.6. Its enzymatic activity is optimal under neutral to slightly alkaline culture conditions of between about pH 7.0 to about 8.5. Production of A. niger phosphatase activity is not repressed by growth on media containing inorganic phosphorus nor is it stimulated by growth on media containing very limited amounts of inorganic phosphorus. The functional activity of this enzyme is stimulated in the presence of magnesium cations (Mg.sup.2+), and to a lesser extent by manganese ions (Mn.sup.2+), but it is significantly inhibited by zinc (Zn.sup.2+) and by calcium (Ca.sup.2+).

L47: Entry 504 of 1063 File: USPT Jul 23, 1996

DOCUMENT-IDENTIFIER: US 5538883 A
TITLE: Maltose-trehalose converting enzyme

DEPR:
Any nutrient culture medium can be used in the invention as long as the microorganisms can grow therein and produce the present enzyme: For example, synthetic- and natural-nutrient culture media can be arbitrarily used. Any carbon-containing substance can be used in the invention as a carbon source as long as it is utilized by the microorganisms: Examples of such a carbon source are saccharides such as glucose, fructose, molasses, trehalose, lactose, sucrose, mannitol, sorbitol, partial starch hydrolysates; and organic acids such as citric acid and succinic acid as well as their salts. The concentrations of these carbon sources in nutrient culture media are appropriately chosen. For example, in the case of using glucose, a preferable concentration is usually 40 w/v % or lower, preferably, 10 w/v % or lower, d.s.b., in view of the growth and proliferation of the microorganisms. The nitrogen sources usable in the invention are, for example, inorganic nitrogen compounds such as ammonium salts and nitrates; and organic nitrogen-containing compounds such as urea, corn steep liquor, casein, peptone, yeast extract and meat extract. The inorganic ingredients usable in the invention are, for example, calcium salts, magnesium salts, potassium salts, sodium salts, phosphates and other salts of manganese, zinc, iron, copper, molybdenum and cobalt.

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L14: Entry 1 of 8 File: USPT Dec 28, 1999

DOCUMENT-IDENTIFIER: US 6007812 A
TITLE: Compound MK7634, process for production thereof, and anthelmintic containing said substance

DEPR:
A fungus producing the MK7634 substance and belonging to imperfect filamentous fungi is cultured in a culture medium containing nutrients which can be utilized by usual microorganisms. As nutrients, those known for use in conventional culture of fungi can be used. For example, as a carbon source, rice, glucose, starch syrup, dextrin, starch, molasses, animal and vegetable oils and the like can be used. As a nitrogen source, soybean meal, wheat germ, corn steep liquor, cotton seed meal, meat extract, peptone, yeast extract, ammonium sulfate, sodium nitrate, urea and the like can be used. It is also effective to add an inorganic salt capable of producing ions such as sodium, potassium, calcium, magnesium, cobalt, chlorine, phosphate and sulfate ions as required. It is also possible to add any organic or inorganic substance enhancing growth of the fungi and production of the MK7634 substance.

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L5: Entry 2 of 4 File: USPT May 24, 1994

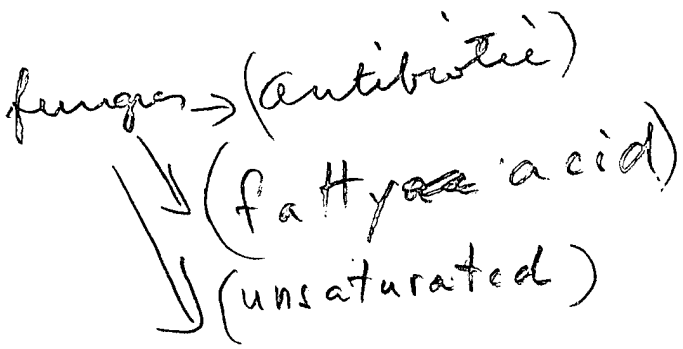
DOCUMENT-IDENTIFIER: US 5314812 A
TITLE: Microbiological process for production of fatty acids having high degree of unsaturation with echinosporangium

DRPR:
For the production of highly unsaturated fatty acids and a lipid containing highly unsaturated fatty acids, spores, mycelia, or a preculture are used as an inoculum for culturing the present strains. The medium used may be a liquid or solid medium. A liquid medium contains as a carbon source, for example, glucose, fructose, xylose, saccharose, maltose, soluble starch, molasses, glycerol, or mannitol. Nitrogen sources include organic substances such as peptone, yeast extract, meat extract, casamino acid, corn steep liquor, and inorganic substances such as sodium nitrate, ammonium nitrate, ammonium sulfate, and the like. If necessary, inorganic salts such as phosphate salts, magnesium sulfate, ferrous sulfate and cupric sulfate, and vitamins may be included in a medium. The concentration of these components is selected so that such components do not adversely affect the growth of the microorganism used. Practically, the concentration of the carbon source is 0.1 to 30% by weight, preferably 1 to 10% by weight, relative to the total weight of the medium. The concentration of the nitrogen source is 0.01 to 5% by weight, preferably 0.1 to 2% by weight, relative to the total weight of the medium.

| DB Name | Query | Hit Count | Set Name |
|---|---|---|---|
| USPT | arachidonic AND Mortierella AND alkaline | 15 | L25 |
| USPT | eicosadienoic AND Mortierella AND alkaline | 1 | L24 |
| USPT | eicosadienoic AND Mortierella AND phosphate AND potassium AND sodium AND magnesium AND calcium | 0 | L23 |
| USPT | eicosadienoic AND Mortierella | 7 | L22 |
| USPT | octadecadienoic AND Mortierella | 10 | L21 |
| USPT | octadecadienoic AND Mortierella AND phosphate AND potassium AND sodium AND magnesium AND calcium | 0 | L20 |
| USPT | eicosayentaenoic AND Mortierella AND phosphate AND potassium AND sodium AND magnesium AND calcium | 0 | L19 |
| USPT | eicosapentaenoic AND Mortierella AND phosphate AND potassium AND sodium AND magnesium AND calcium | 1 | L18 |
| USPT | eicosapentaenoic AND Mortierella | 36 | L17 |
| USPT | L15 AND phosphate AND potassium AND sodium AND magnesium AND calcium AND Mortierella | 2 | L16 |
| USPT | "fatty acid" AND arachidonic | 3017 | L15 |
| USPT | L13 AND phosphate AND potassium AND sodium AND magnesium AND calcium | 15 | L14 |
| USPT | "unsaturated" AND Mortierella | 60 | L13 |
| USPT | L11 AND calcium | 16 | L12 |
| USPT | L10 AND magnesium | 20 | L11 |
| USPT | L9 AND sodium | 29 | L10 |
| USPT | L8 AND potassium | 35 | L9 |
| USPT | L7 AND phosphate | 48 | L8 |
| USPT | "fatty acid" AND Mortierella | 83 | L7 |
| USPT | "fatty acid" | 90239 | L6 |
| USPT | L2 SAME phosphate | 4 | L5 |
| USPT | L2 SAME M | 5 | L4 |
| USPT | L2 SAME ions | 0 | L3 |
| USPT | "unsaturated fatty acids" SAME cultur$ | 114 | L2 |
| USPT | "unsaturated fatty acids" AND cultur$ | 1229 | L1 |

| DB Name | Query | Hit Count | Set Name |
|---|---|---|---|
| USPT | l13 AND (calcium) | 6 | L14 |
| USPT | l12 and culture | 10 | L13 |
| USPT | L3 SAME (sodium or potassium) | 53 | L12 |
| USPT | L3 AND (sodium or potassium) | 284 | L11 |
| USPT | L3 SAME produce | 41 | L10 |
| USPT | l8 AND produce | 28 | L9 |
| USPT | l7 and culture | 35 | L8 |
| USPT | l6 and potassium | 70 | L7 |
| USPT | l5 and magnesium | 73 | L6 |
| USPT | l4 and sodium | 125 | L5 |
| USPT | l3 and calcium | 144 | L4 |
| USPT | fungus SAME "fatty acid" | 351 | L3 |
| USPT | fungus AND "fatty acid" | 6599 | L2 |
| USPT | fungus AND antibiotic | 6342 | L1 |

| DB Name | Query | Hit Count | Set Name |
|---|---|---|---|
| USPT | l28 AND "culture medium" | 6 | L29 |
| USPT | l27 AND calcium | 84 | L28 |
| USPT | l26 AND magnesium | 109 | L27 |
| USPT | l25 AND sodium | 160 | L26 |
| USPT | phosphate AND postassium | 166 | L25 |
| JPAB,EPAB,DWPI,TDBD | l20 SAME culture | 0 | L24 |
| JPAB,EPAB,DWPI,TDBD | l21 | 0 | L23 |
| USPT | l20 SAME culture | 16 | L22 |
| USPT | l20 AND culture | 88 | L21 |
| USPT | l18 SAME ion | 1176 | L20 |
| USPT | l18 AND ion | 5661 | L19 |
| USPT | adjust SAME concentrat$ | 13247 | L18 |
| JPAB,EPAB,DWPI,TDBD | L8 SAME ion$ | 0 | L17 |
| JPAB,EPAB,DWPI,TDBD | L8 SAME ion$ | 0 | L16 |
| JPAB,EPAB,DWPI,TDBD | L8 SAME ion$ | 0 | L15 |
| JPAB,EPAB,DWPI,TDBD | L8 SAME ion$ | 0 | L14 |
| JPAB,EPAB,DWPI,TDBD | L8 SAME ion$ | 0 | L13 |
| USPT | L8 SAME ion$ | 8 | L12 |
| USPT | L8 SAME ion$ | 8 | L11 |
| USPT | L8 SAME ion$ | 3 | L10 |
| USPT | L8 AND ions | 109 | L9 |
| USPT | L5 SAME culture | 260 | L8 |
| USPT | l5 AND cultur$ | 1333 | L7 |
| USPT | l5 AND culture | 1306 | L6 |
| USPT | "filamentous fungus" | 1422 | L5 |
| USPT | fungus | 24975 | L4 |
| USPT | "fillamentous fungus" | 0 | L3 |
| USPT | "fillamentous fungus" | 0 | L2 |
| USPT | "fillamentous fungus" | 0 | L1 |



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**Search Results -**

| Terms | Documents |
|---|---|
| L37 SAME "filamentous fungus" | 6 |

**Database:** US Patents Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Refine Search: L5 SAME cultur$ Clear

**Search History**

**Today's Date: 7/27/2000**

| DB Name | Query | Hit Count | Set Name |
|---|---|---|---|
| JPAB,EPAB,DWPI,TDBD | L37 SAME "filamentous fungus" | 6 | L39 |
| JPAB,EPAB,DWPI,TDBD | ions SAME cultur$ SAME phosphate SAME potassium SAME sodium SAME magnesium SAME calcium SAME "filamentous fungus" calcium | 122374 | L38 |
| JPAB,EPAB,DWPI,TDBD | ions SAME cultur$ SAME phosphate SAME potassium SAME sodium SAME magnesium SAME calcium calcium | 122374 | L37 |
| USPT | "mycelial morphology" SAME ions | 0 | L36 |
| USPT | "mycelial morphology" | 8 | L35 |
| USPT | L32 SAME "filamentous fungus" | 1 | L34 |
| USPT | L32 SAME Mortierella | 1 | L33 |
| USPT | ions SAME cultur$ SAME phosphate SAME potassium SAME sodium SAME magnesium | 307 | L32 |

| | | | |
|---|---|---|---|
| | SAME calcium | | |
| USPT | ions SAME cultur$ SAME phosphate SAME potassium SAME sodium SAME magnesium | 321 | L31 |
| USPT | ions SAME cultur$ SAME phosphate SAME potassium SAME sodium | 432 | L30 |
| USPT | ions SAME cultur$ SAME phosphate SAME potassium | 507 | L29 |
| USPT | ions SAME cultur$ SAME phosphate | 989 | L28 |
| USPT | ions SAME cultur$ | 5536 | L27 |
| USPT | cultur$ SAME Mortierella SAME "unsaturated fatty acid" | 5 | L26 |
| USPT | cultur$ SAME Mortierella | 68 | L25 |
| USPT | 5997913 | 1 | L24 |
| USPT | ions SAME fermentation SAME cultur$ SAME fungus | 4 | L23 |
| USPT | ions SAME fermentation SAME cultur$ | 458 | L22 |
| USPT | ions SAME fermentation | 1569 | L21 |
| USPT | L5 SAME fungus SAME ion | 0 | L20 |
| USPT | L5 SAME fungus | 25 | L19 |
| USPT,JPAB,EPAB,DWPI,TDBD | L5 SAME "filamentous fungus" | 1 | L18 |
| USPT | "fermentation" SAME "Mortierella" | 28 | L17 |
| USPT | L5 SAME "ions" SAME phosphate | 6 | L16 |
| USPT | L5 SAME "ions" | 126 | L15 |
| JPAB,EPAB,DWPI,TDBD | "Mortierella" SAME "ions" | 4 | L14 |
| USPT | "Mortierella" SAME "ions" | 2 | L13 |
| USPT | "Mortierella" | 174 | L12 |
| USPT | "ions" SAME "concentrations" | 54613 | L11 |
| USPT | "fermentation" SAME "Mortierella" SAME "ions" | 0 | L10 |
| USPT | "fermentation" SAME "Mortierella" | 28 | L9 |
| USPT | "unsaturated fatty acids" SAME "Mortierella" SAME "concentrations" | 1 | L8 |
| USPT | "unsaturated fatty acids" SAME "Mortierella" SAME "ions" | 0 | L7 |
| USPT | "unsaturated fatty acids" SAME "Mortierella" | 13 | L6 |
| USPT | "unsaturated fatty acids" | 9799 | L5 |
| USPT | "filamentous fungus" SAME "concentrations" SAME "ions" | 4 | L4 |
| USPT | "filamentous fungus" SAME "concentrations" SAME "phosphate ions" | 0 | L3 |

| | | | |
|---|---|---|---|
| USPT | "filamentous fungus" SAME "concentrations" | 115 | L2 |
| USPT | "filamentous fungus" | 1422 | L1 |